TISSUE ENGINEERING
Volume 2, Number 1, 1996
Mary Ann Liebert, Inc.

The Effects of Dynamic Compressive Loading on Biodegradable Implants of 50–50% Polylactic Acid–Polyglycolic Acid

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ABSTRACT

Biodegradable implants that release growth factors or other bioactive agents in a controlled manner are investigated to enhance the repair of musculoskeletal tissues. In this study, the *in vitro* release characteristics and mechanical properties of a 50:50 polylactic acid/polyglycolic acid two phase implant were examined over a 6-week period under no-load conditions or under a cyclic compressive load, such as that experienced when walking slowly during rehabilitation. The results demonstrated that a cyclic compressive load significantly slows the decrease of molecular chain size during the first week, significantly increases protein release for the first 2–3 weeks, and significantly stiffens the implant for the first 3 weeks. It was also shown that protein release is initially high and steadily decreases with time until the molecular weight declines to about 20% of its original value (approximately 4 weeks). Once this threshold is reached, increased protein release, surface deformation, and mass loss occurs. This study also showed that dynamic loading and the environment in which an implant is placed affect its biodegradation. Therefore, it may be essential that *in vitro* degradation studies of these or similar implants include a dynamic functional environment.

INTRODUCTION

BIODEGRADABLE IMPLANTS are often used for the controlled release of drugs *in vivo* over prolonged periods of time. For instance, drugs or other bioactive agents are incorporated in biodegradable polymers that are surgically placed in the desired compartment of the body. As the implants degrade over time, they release the agent in the localized area. Controlled release is actively pursued in orthopedics to enhance repair of musculoskeletal tissues, using growth factors that induce repair of soft and hard tissues.[1–10] A porous biodegradable implant can release such growth factors while providing a mechanical scaffold for new tissue. However, for effective healing, the implants should have a steady release of growth factor and a simultaneous decrease in mechanical properties without inducing an immune response. An accelerated degradation rate can result in a high concentration of degradation by-products, causing toxicity and inflammation.[11,12] An earlier study by our group has determined that the *in vitro* release characteristics and

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mechanical properties of biodegradable implants developed for the repair of osteochondral defects are suitable under no-load conditions, yielding a progressive sigmoidal protein release pattern and a linear degradation of mechanical properties over a 10-week period.[13] However, it is unlikely that patients will be bedridden for such a long period, especially because the application of mechanical stress often helps strengthen the articular cartilage through mechanical conditioning.[14] Therefore, it is desirable to determine the release characteristics and mechanical properties of the osteochondral implant when a dynamic compressive load is applied, such as that experienced in hip or knee joints when walking.

Previous studies have yielded inconclusive results on the effects of a dynamic load on biodegradable materials. Cyclic tensile loading was shown to have no significant effect on the biodegradation of a polylactic acid–polyglycolic acid (PLA–PGA) copolymer.[15] However, cyclic flexural loading was shown to have a significant effect on the protein release characteristics of such a copolymer.[16] In addition, a dynamic flexural load was shown to have a significant effect on the degradation of mechanical properties of poly(ortho)esters.[17]

The PLA–PGA families have been widely used for fabricating implantable devices and have proven to be relatively biocompatible.[4,18] The structure–property relationships of these materials have been investigated in detail.[19–21] Additionally, their biodegradation characteristics have been studied extensively.[4,12,13,22–32] The effect of ultrasound irradiation on a PLA–PGA polymer has also been studied.[33,34] Ultrasound irradiation applies a microscopic mechanical stress to the polymer, straining the molecular chains and making them more susceptible to hydrolysis. Ultrasonic energy also provides an increase in diffusion of water into the implant and accelerated transport of degradation products out of the implant, resulting in enhanced mass loss, molecular weight loss, and protein release.[33] Consequently, ultrasound irradiation results in a 3-fold increase in protein release compared to nonloaded biodegradation of implants fabricated from a 50:50 PLA–PGA copolymer.[33] The present study was performed to investigate the *in vitro* effects of a dynamic compressive load on a polymeric implant containing a test protein. A biodegradable copolymer of PLA and PGA was used as the implant material. This type of implant has been used previously in osteochondral repair.[9,10]

MATERIALS AND METHODS

Implants

A total of 132 two phase biodegradable implants, simulating an osteochondral plug of subchondral bone with attached articular cartilage, were produced by dissolving a 50:50 poly(DL-lactide coglycolide) copolymer (inherent viscosity 0.71 dl/g, Birmingham Polymers, Inc., Birmingham, AL) in acetone and then precipitating it in ethanol to obtain a gummy precipitate.[9,10,13] The precipitate was then cast into a cyclindrial shape (7 × 7 mm) in a custom mold while applying vacuum and temperature (37°C) to obtain a porous structure (porosity approximately 65%). The bone phase was heated an additional 24 h at 47°C to create a stiffer structure than the cartilage phase (bone stiffness = 10 cartilage stiffness). The implants were freeze dried and stored until ready for testing. For this study, soybean trypsin inhibitor (TI type I-S, Sigma Chemical Co., St. Louis, MO) was added to the cartilage phase of half of the implants while the copolymer was in solution with acetone. TI is a molecule with molecular weight and solubility comparable to that of bone morphogenic protein (BMP)[35] at a fraction of the cost, and results of the TI testing are expected to closely resemble those of BMP.[13,36] Approximately 3.5 mg of TI was added to each of 66 implants. The TI may be trapped in the matrix formed by the copolymer during precipitation.[13] The other half of the implants, 66, were produced without adding TI.

The implants were weighed and divided into five groups to test four *in vitro* conditions: (1) nonloaded biodegradation without TI, (2) nonloaded biodegradation with TI, (3) dynamic biodegradation without TI, and (4) dynamic biodegradation with TI (Table 1). The fifth group was a control group of 12 implants, six with TI and six without TI, that were dried and stored under vacuum for later analysis without undergoing biodegradation.

Nonloaded Degradation

To test nonloaded biodegradation, 60 implants (30 with TI and 30 without TI) were placed in individual 20-ml glass scintillation vials containing 5 ml of phosphate-buffered saline (PBS) at 37°C for various time

TABLE 1. EXPERIMENTAL DESIGN

	Nonloaded biodegradation			*Dynamic biodegradation*		
Time (days)	*Implants without TI (number of implants removed)*	*Implants with TI (number of implants removed)*	*Implants with TI (number of solution samples)*	*Implants without TI (number of implants removed)*	*Implants with TI (number of implants removed)*	*Implants with TI (number of solution samples)*
0	6 (control)	6 (control)				
3.5			6			6
7.0	6	6	6	6	6	6
10.5			6			6
14.0	6	6	6	6	6	6
17.5			6			6
21.0	6	6	6	6	6	6
24.5			6			6
28.0	6	6	6	6	6	6
31.5			6			6
35.0			6			6
38.5			6			6
42.0	6	6	6	6	6	6
Total	36	36	132	30	30	132

intervals. Inside the vials, the implants were supported in a confined compression configuration by a surrounding porous Teflon ring to represent the surrounding cartilage *in vivo* and to provide an equivalent basepoint for comparison of the dynamically loaded samples. For the 30 implants containing TI, samples of the PBS were withdrawn from the scintillation vials every 3.5 days and frozen for later analysis for the amount of protein released from the implant. The solution was briefly vortexed for 5 sec before aspirating the samples to ensure a uniform and representative protein concentration. Each vial was vortexed the same amount of time at the same frequency (5 Hz) to avoid inducing a variation in degradation as a result of the vortex operation. Because hydrolytic breakdown of the implants releases hydrogen ions into the buffered saline solution, the PBS solution in all 60 scintillation vials was replaced every 3.5 days to ensure a pH of 7.4. Every 7 days for the first 4 weeks, 12 implants (6 with TI, 6 without TI) were removed, dried, and stored for later analysis of the molecular weight, mass, mechanical properties, and surface morphology. The final 12 implants were removed from the test fixture at the end of the 6-week test period, dried, and stored for later analysis.

Dynamic Degradation

To test the effects of dynamic loading on biodegradation, 60 implants (30 with TI and 30 without TI) were placed in individual 20-ml glass scintillation vials containing 5 ml of PBS at 37°C. Inside each vial, the implant was supported in a confined compression configuration by a surrounding porous ring to constrain the sides of the implant during loading. Daily, the scintillation vials were secured on custom piston apparatuses (Fig. 1) and a cyclic load was applied to the top of each implant for 90 min. Each piston apparatus consisted of a 6-mm-diameter Teflon piston attached to a stainless-steel air cyclinder. For each piston apparatus, a hole was centered and drilled in the screw lid of a scintillation vial, and the lid was secured to the body of the air cylinder body with a nut. By attaching the vial lid to the air cylinder, the sample vials could easily be installed and removed from the piston apparatuses. Thus, the dynamically loaded sample vials could be easily exchanged and rotated through the 12-piston apparatuses for 90 min of loading each day to allow more economical use of the loading hardware. An air cylinder with a double acting piston with the reverse port exposed to atmosphere was chosen instead of a single acting piston to allow a

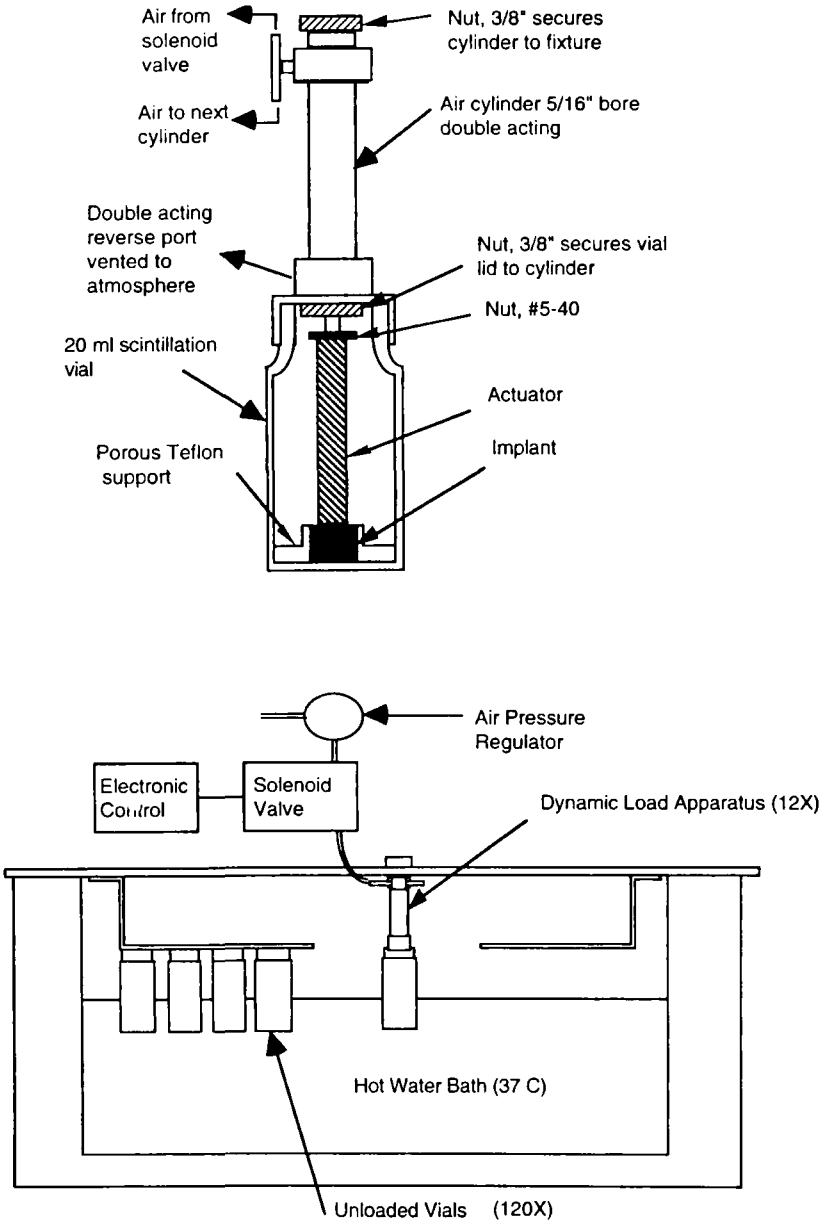

FIG. 1. Dynamic loading fixture. The dynamically loaded implants were rotated through the 12 dynamic loading apparatus for 90 min each day. The air cylinder applies an area equivalent constant force load of 1× body weight every 2 sec. A pressure regulator precisely controls the air pressure to the cylinder, resulting in a constant force load imparted on the implant. Control implants degraded under nonloaded conditions.

constant force to be applied to the piston without an internal spring resistance force. Thus, the only force resisting compressive motion of the piston is the viscoelastic force of the implant. A pressure regulator accurately controls the pressure seen by the piston, yielding a constant force load. The air flow to the air cylinder is regulated by an electronically controlled solenoid valve. When the air pressure to the air cylinder is turned off and the piston is vented to atmosphere by the solenoid valve, the viscoelastic properties of the implant allow it to recover and push the piston in reverse. A preliminary test using a load cell allowed the cyclic compressive force generated by the air cylinder to be assessed. The preliminary test showed that the force generated by the air cylinders is constant and repeatable. The preliminary test also determined the supply air pressure that would provide sufficient air flow through the solenoid valve to yield the desired pressure at the air cylinders for the loading cycle of the experiment. Thus, the upstream line pressure could be regulated at a level that provided the desired load during the study.

A 3.72 N load was applied to the top of each implant for 0.1 sec at a frequency of 0.5 Hz for 2700 cycles daily. The frequency, load, and duration simulate the area equivalent load of 1× body weight (700 N) seen in the knee during slow walking by a patient undergoing rehabilitation. The load represented the equilibrium forces seen in the knee using an average aggregate modulus of 0.6 MPa.[37–42] Because the air cylinders have a slight frictional force, the test load applied to the implants included a tare load slightly greater than the maximal cylinder frictional load. By including the tare load, the effect of frictional load variation on implant loading was eliminated. The double acting cylinder and tare load allow the piston to reverse to an equilibrium point that is dependent solely on the viscoelastic properties of the implant, preventing a rapid reversal and "jackhammer" impact effect on the next loading cycle. The number of daily cycles was recommended for wear testing of orthopedic joints.[43] The load duration was determined from preliminary tests that assessed the duration that would minimize creep to avoid damage to this particular implant for the conditions stated above. The preliminary tests were performed by using an automated stress and creep compressing device built in our laboratory that applied a cyclic step load to the implant and measured the resulting displacement. The device allowed the load duration to be varied to determine the maximum duration that would prevent the implant from excessive creeping when newly installed. An *in vitro* load duration greater than 0.1 sec causes this particular implant with initial material properties to be successively crushed by the cyclical load when applied for several hundred cycles. Although the 0.1 sec load duration may be shorter than that seen when walking, it is assumed that the implant will not be initially crushed under *in vivo* conditions because the surrounding cartilage will help limit the compression of the implant. Future studies will determine if this assumption is correct, but for the purposes of this study, an initial noncollapsing compressive load was desired.

For the 30 implants containing TI, solution samples were obtained in a manner, number, and frequency identical to that previously described in the nonloaded degradation section. In addition, implant samples were similarly obtained in number, manner, and frequency as previously described in the nonloaded degradation section. The dynamic test was allowed to run for 6 weeks, at which time the molecular weight of the polymer was expected to have decreased to nonmeasurable levels and the protein released.[13] At the end of the 6-week period, the remaining 12 implants were removed, dried, and stored for later analysis.

Protein Release Analysis

Temporal changes in protein release were analyzed as a function of TI concentration in the PBS solution samples. The solution samples were thawed at room temperature for 1 h, and TI concentration was estimated using a bichonic acid protein assay (MicroBCA, Pierce, Rockford, IL) in 100-μl aliquots and an ELISA reader.

Mass and Molecular Weight Analysis

The sample implants were weighed to obtain changes in mass over time. Changes in polymer chain size were obtained by measuring the weight average molecular weight of the implant samples using gel permeation chromatography. Specimens (approximately 3 mg) were sliced from the dried implants, placed in 1.5-ml microcentrifuge vials, and dissolved in 1 ml chloroform. Separate specimens were obtained for the bone phase and cartilage phase of each implant. Using a 5-ml syringe, the solution in each vial was then aspirated through a micropore filter (0.45 μm) to filter out any remaining TI or unwanted contaminants. A new filter and syringe were used for each sample to ensure that the samples were not mixed and contaminated. After aspiration, each sample solution was transferred to an individual clean microcentrifuge vial for storage. A solution sample (100 μl) was then again aspirated into a microsyringe and injected into the gel permeation chromatographer where it was carried by the mobile phase (chloroform) through two serial columns with a linear range of pore sizes, previously calibrated with polystyrene standards in a chloroform mobile phase (Waters μStyragel HT 35554, Millipore Corporation, Milford, MA).

Mechanical Properties Analysis

Mechanical properties of the implants were measured using an automated creep indentation apparatus developed in our laboratory.[38] Normal saline was added to each implant and they were allowed to rehy-

drate for 24 h. The implant was then attached to a specimen holder with cyanoacrylate adhesive such that the cartilage phase was the free surface. A 2.0-mm-diameter pin was placed on the desired test area of the cartilage phase. The specimen holder was maneuvered until the pin was aligned with the indenter to ensure a perpendicular alignment between the implant and indenter. The pin was then removed and the test chamber was filled with normal saline, immersing the implant. A tare load of 0.1 N was applied through a 2.0-mm-diameter porous rigid indenter tip and the implant was allowed to creep for 30 min. A test load of 0.5 N was then applied through the tip, and the implant was allowed to creep for 60 min. The amount of axial surface creep deformation was measured and recorded.

Gross Morphology Assessment

The implants were inspected every 3.5 days during the test when the PBS solution was exchanged. Features of their appearance and any changes were recorded. After freeze drying, the implant samples were photographed at 2.5× magnification to document the gross morphologic changes.

Statistical Analysis

The following quantitative properties were measured for each implant: mass, weight average molecular weight, and axial surface deformation. The percent of released protein in each solution sample was calculated using the measured TI concentration, the known PBS solution volume, and the known initial protein mass contained in each implant. These properties were compiled as mean ± standard deviation and were examined statistically with analysis of variance and unpaired Student's t test as needed. The data of the dynamically loaded condition were compared with the data of the nonloaded condition at each sample time point to determine if there was statistical significance between the means ($p < 0.05$).

RESULTS

Figure 2A shows the degradation of the weight average molecular weight of the cartilage phase of the implant over a 4-week period. Initially, the mean molecular weight of the cartilage phase was 47 kDa. In the first week, there was a significant difference in the molecular weight of the dynamically loaded implants containing protein versus the corresponding nonloaded implants, with the cyclically loaded implants degrading slower than the nonloaded implants. In the following weeks, the molecular weight of the implants under both conditions declined in an approximately linear manner, with no significant difference between the two means. However, results showed a trend indicating that the nonloaded samples degraded faster than the dynamically loaded samples. By the end of week 6, the material had degraded to an extent that separate cartilage and bone phases could not be distinguished from each other.

The degradation of the weight average molecular weight of the bone portion of the implant is shown in Figure 2B. The results closely resemble those of the cartilage phase. Initially, the mean molecular weight was 47 kDa. At the end of the first week, the molecular weight of the dynamically loaded implant was significantly higher than the nonloaded samples. The molecular weight decreased almost linearly in the following 5 weeks, with the nonloaded samples maintaining lower molecular weight than the dynamically loaded samples.

The mass of the implants remained almost constant during the first 3 weeks. The rate of mass loss increased during week 4, and by the end of week 6, the mass of the samples had declined to about 88% of the original. There were no significant differences in the mass between nonloaded and dynamically loaded samples during the 6-week test period, but the results show a trend suggesting that the dynamically loaded samples began to lose mass at a greater rate than the nonloaded ones after the third week.

The temporal changes in protein release from the cartilage phase over the 6-week test period are shown in Figure 3. Although the amount of protein released during the first 17 days declined with time for both loading conditions, the dynamically loaded implants released significantly more protein than the nonloaded implants. From days 17 to 28, the protein release from the nonloaded implant was almost constant while the protein release from the dynamically loaded sample continued to decline, resulting in significantly lower

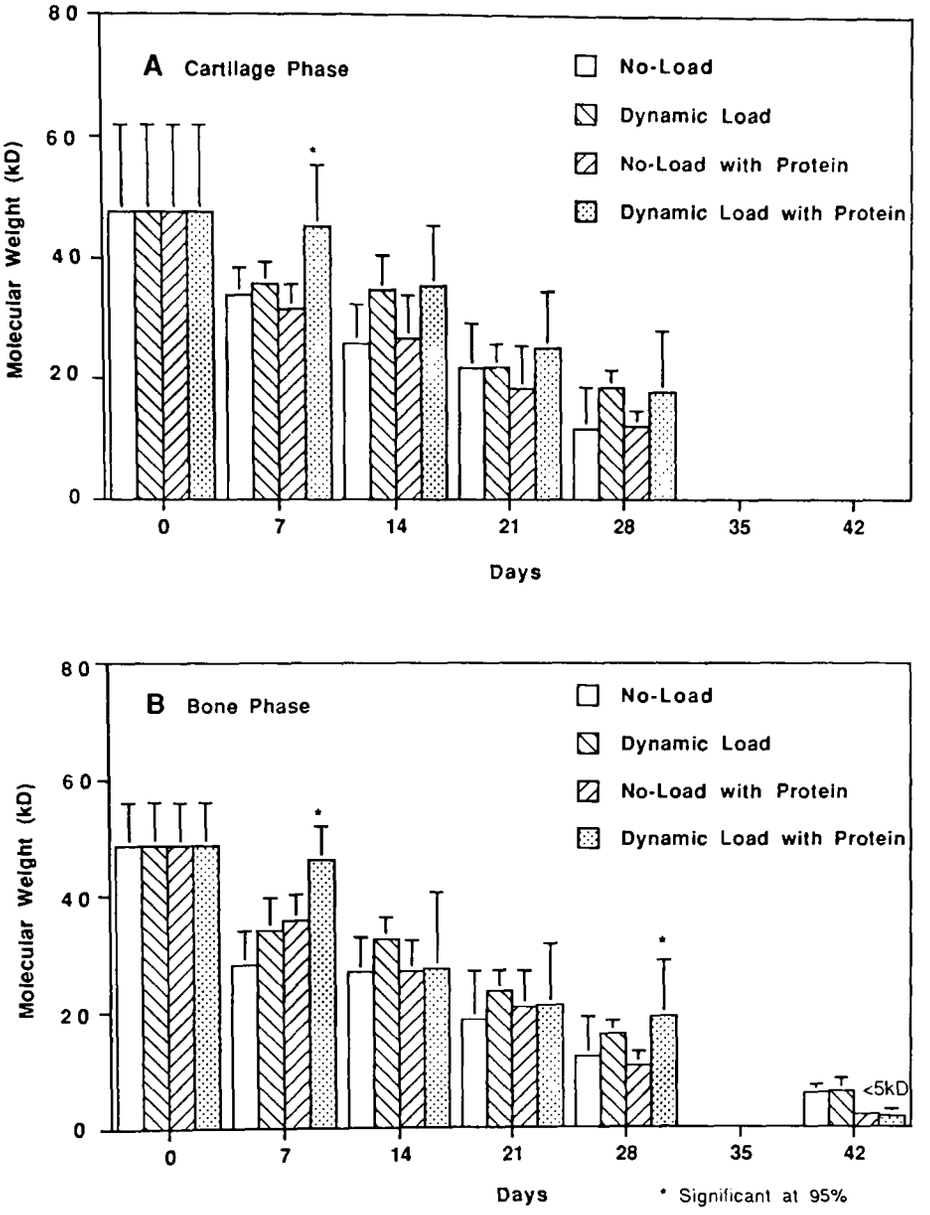

FIG. 2. Weight average molecular weight of the two-phase implant. Unpaired Student's t tests $p < 0.05$ at each sample time point show that the molecular weight of the cartilage phase (A) of the dynamically loaded implant declines significantly slower in the first week than the implant under nonloaded conditions. Similar results are seen in the bone phase (B), where the molecular weight of the dynamically loaded implant decreases significantly slower in weeks 1 and 4 than the corresponding non-loaded implant. Results shown as mean ±SD.

release. For days 31 to 42, the protein release increased with time for both loading conditions, with no significant difference between them. The cumulative protein release is compared in Figure 4. At the end of 6 weeks, the dynamically loaded samples had released approximately 43% of their initial load of TI, compared to approximately 28% for the nonloaded samples.

The temporal variation in the axial surface deformation of the cartilage phase of the implants containing protein is shown over a 4-week period in Figure 5. Results show significantly less deformation for the dynamically loaded samples than the nonloaded implants at each time point during the first 3 weeks. The deformation was minimized at the end of week 2, and began to increase substantially after week 3. Beyond 4 weeks, deformation data were not obtained because of significant loss of shape and rigidity.

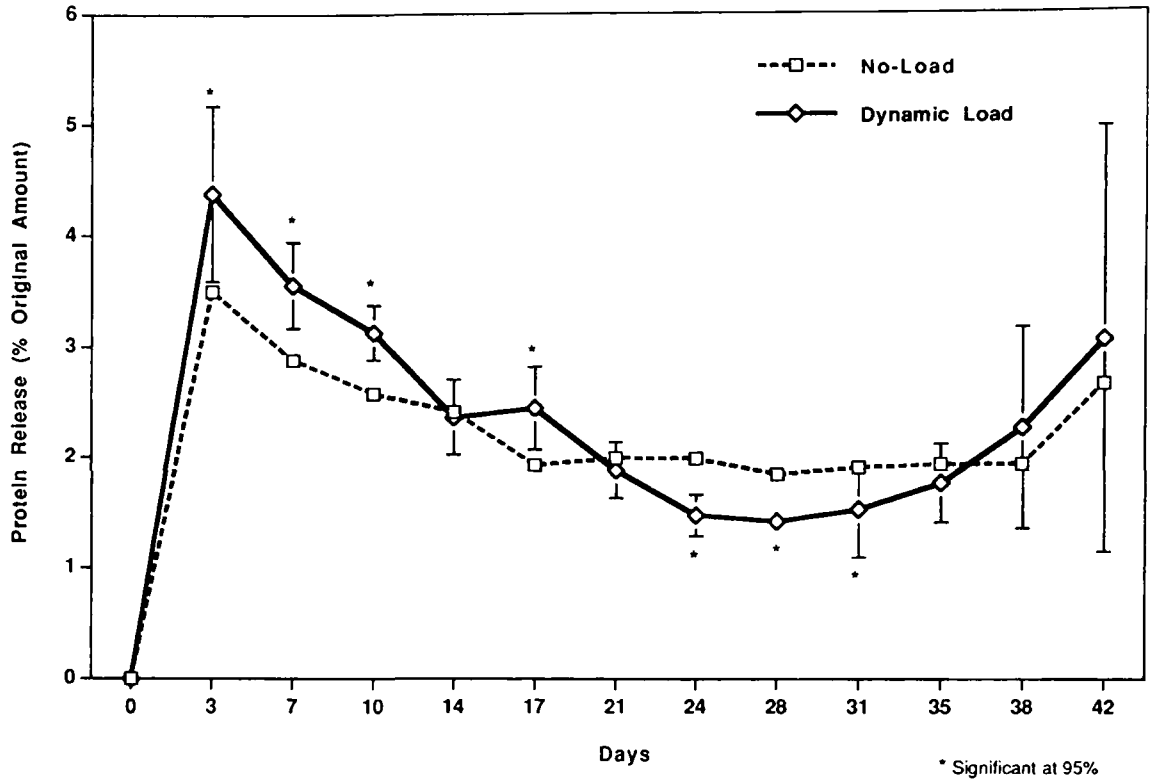

FIG. 3. Protein release decreases for the first 28 days. In comparison with the nonloaded implants ($p < 0.05$) at each sample time point, the dynamically loaded implants show significantly greater protein release for the first 17 days, significantly lower protein release from day 17 to 31, and increasing release from day 31 to 42 ($p < 0.05$). Results shown as mean ±SD.

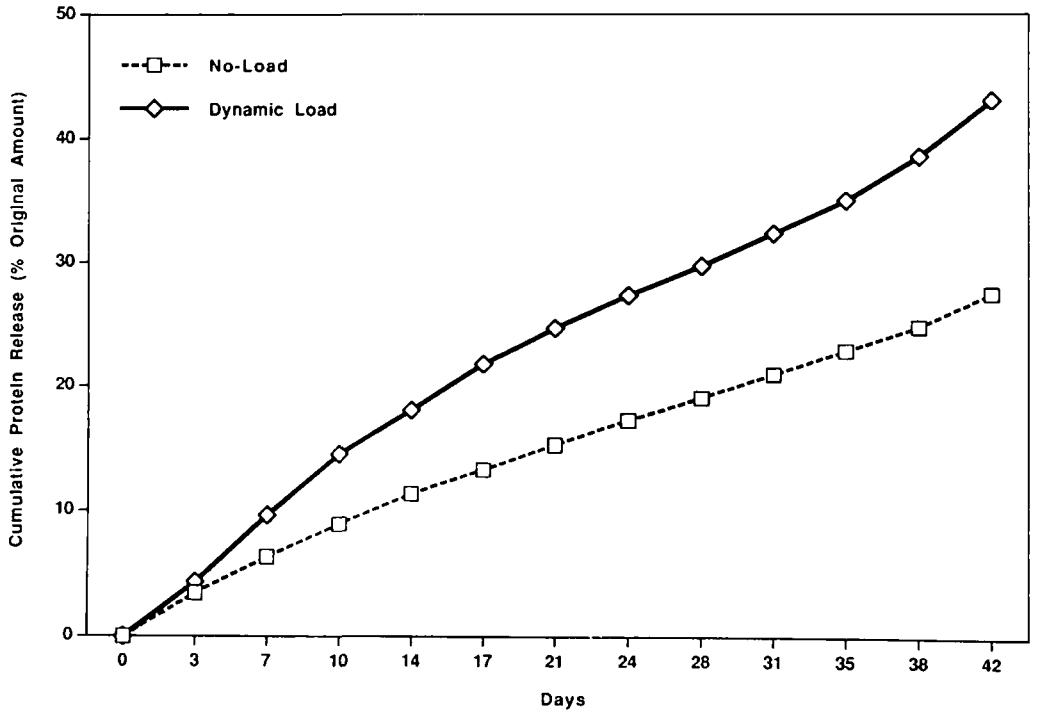

FIG. 4. Cumulative protein release is steady throughout the 6-week test period. The dynamically loaded samples release 15.5% more protein than the implants under nonloaded conditions by the end of the sixth week. Shown are the mean values.

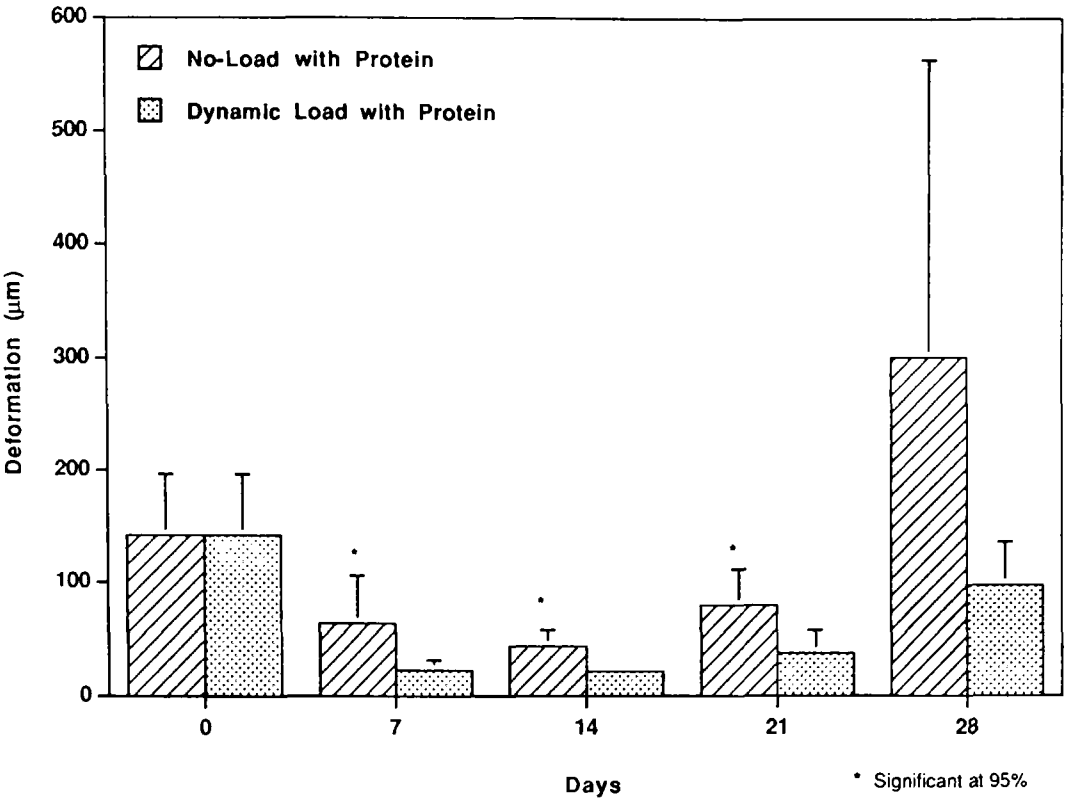

FIG. 5. Implant surface deformation. Compared with the nonloaded condition at each sample time point ($p < 0.05$), dynamic loading significantly stiffens the implants ($p < 0.05$), with a maximum stiffness at the end of the second week. Results shown as mean ±SD.

Gross morphologic changes were observed during the 6-week period. Initially, the implants containing protein were smooth and cylindrical, as shown in Figure 6. By the end of the first week, all implants appeared chalky white, and the cyclically loaded implants became shorter than their nonloaded counterparts due to plastic deformation imparted by the compressive load. By the end of the second week, increased plastic deformation was observed in all dynamically loaded implants; the nonloaded implants exhibited more pronounced surface porosity. During weeks 3 and 4, the implants began to soften, and the cyclically loaded samples began to change shape via extrusion through the bottom and porous channels of the Teflon support. In weeks 3 and 4, the nonloaded samples maintained their external shape, but began to have a central hollow core that projected up from the bottom of the implant. By the end of the sixth week, the implants had become gummy and significant morphologic changes could be observed (Fig. 6). The dynamically loaded implants had been compressed into pancakes that had no resemblance to their initial structure. The nonloaded implants retained more of their initial shape, but were still emaciated and deformed.

DISCUSSION

Mechanical stresses, as may be expected in orthopedic applications, are likely to alter the mechanical behavior as well as the drug or protein release kinetics of biodegradable devices. The results of this study clearly support this assertion. The implants subjected to dynamic compressive loading underwent a reduction in volume and became more compact compared to the nonloaded specimens. This change is not surprising because external stresses can potentially cause a collapse of the pores in the polymer matrix. The resulting less porous structure would present a smaller surface area for hydrolysis, which would reduce the rate of degradation. The lower molecular weight loss of the loaded specimens compared to the nonloaded specimens (Fig. 2), although not statistically significant, is suggestive of this phenomenon.

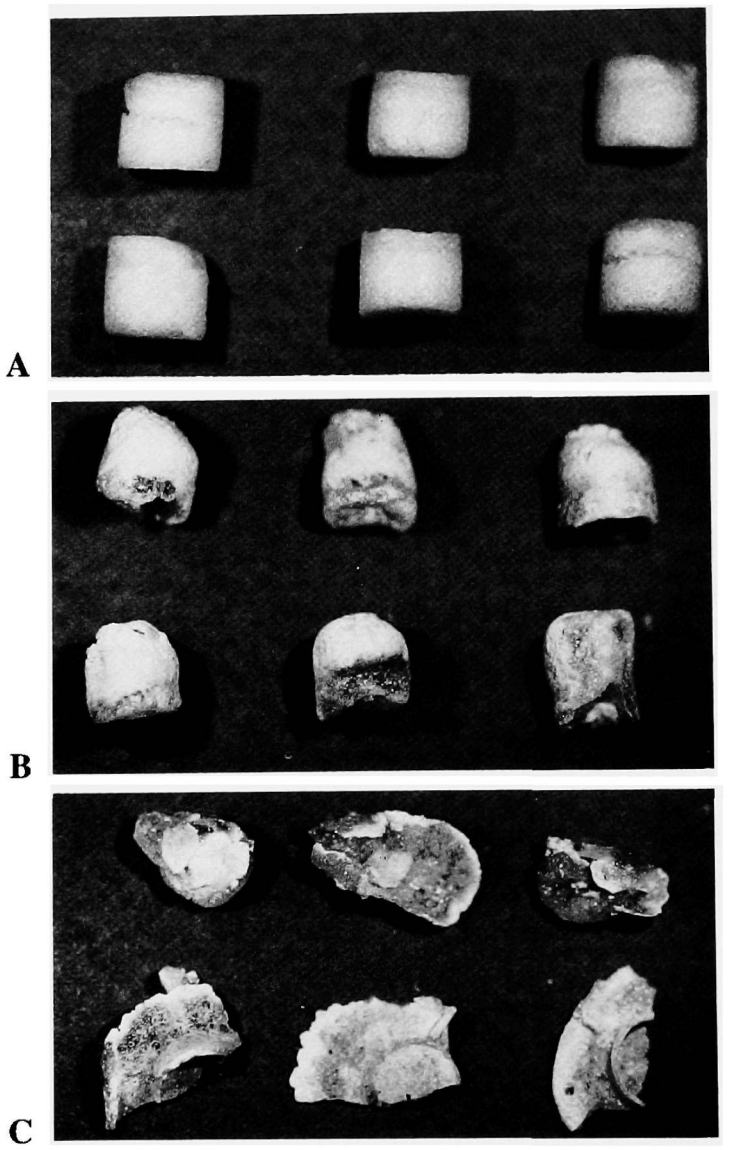

FIG. 6. Implant gross morphological changes. Specimens shown have been freeze-dried and photographed at 2.5× magnification. (A) Day 0, (B) day 42 of nonloaded degradation, (C) day 42 of degradation of dynamically loaded implants. Dynamic loading significantly deforms the implants by the end of the sixth week.

As shown in Figure 5, the dynamically loaded specimens exhibited a lower deformation than the nonloaded implants when subjected to a compressive test load. This is a manifestation of the higher stiffness that a less porous material would be expected to exhibit. Thus, the changes in surface deformation and morphology suggest that the compressive loading initially collapses and stiffens the implant. However, as hydrolysis breaks down the polymer chains a threshold is reached beyond which the polymer may no longer accommodate the mechanical load and begins to yield substantially.

Agrawal et al.[33] reported a significant increase in protein release from PLA–PGA implants subjected to ultrasound irradiation. In the present work the application of cyclic stresses had a similar impact on the protein release characteristics of the PLA–PGA implant. As shown in Figure 4, this implant provided a steady release of protein throughout the test for both dynamic load and no-load conditions, but the former substantially accelerated the cumulative protein release. Thus at the end of 6 weeks, dynamic loading resulted in the release of 15% more protein compared to the nonloaded implants. To understand this phenomenon one must first consider the protein release patterns for both conditions. The overall trends in each case were

similar: an initial high elution rate, decreasing to a minimum at approximately 4 weeks, followed by an increase up to the end of the study. Thus, the release characteristics are perhaps diffusion controlled initially, and the protein loosely held in the polymer matrix is released as water invades the pores of the implant. By week 4, the molecular weight of the cartilage phase decreases substantially and the average polymer molecular chain is similar in size to the protein molecule. Further polymer breakdown facilitates release of the protein intimately associated with the polymer. As the average size of the polymeric molecules decreases it becomes progressively easier for them to diffuse out of the matrix leading to an increased rate of mass loss and a concomitant increase in protein release (Fig. 3).

As shown in Figure 3, dynamic loading of the implant increases the initial rate of protein elution compared to the nonloaded implants. Such an increase may arise from the enhanced fluid flow in and out of the implant due to the cyclic loading. However, as this reservoir of loosely associated protein is rapidly depleted, the elution rate decreases and falls below that of the nonloaded control implants from approximately 3 to 5 weeks. As described earlier, following this time period protein release begins to be dominated by polymer degradation and consequently the elution rate increases. Once again, the "pumping" action associated with the dynamic loading may assist in the egress of the protein, although this effect results only in a marginal and statistically insignificant increase in both mass loss and the elution rate compared to the controls. This phenomenon is further complicated by the fact that dynamic loading is likely to collapse the pores of the implant and consequently decrease its rate of degradation.

Interestingly this investigation, in combination with a previous study,[6] demonstrates that implant configuration also has a substantial effect on early protein release. In this study, two-phase implants were used. All of the protein was concentrated at the top of the implant in the cartilage phase. In contrast, the previous study used a single phase implant in which an equal amount of protein was dispersed in a larger volume of material.[6] Consequently, the cartilage phase of the present implant had a higher initial protein concentration than the earlier single phase implant. The results of the two studies confirm that the two-phase implant initially releases more protein perhaps due to the higher concentration gradient compared to the single phase implant.

The environment in which an implant is placed can affect its biodegradation and the resulting morphology. Rather than floating unconstrained in solution, the implants in this study were partially constrained on the sides and bottom by a porous Teflon support. This constrained configuration may be a closer representation of *in vivo* conditions where the implant would be surrounded by bone and hyaline cartilage. The constraining environment, however, changes the local fluid flow surrounding the implant, possibly slowing diffusion on all surfaces except the top cartilage phase. As hydrolytic breakdown occurs the implant releases acidic by-products. Where diffusion is restricted by a constraining environment, these by-products can accumulate within the implant and locally lower the pH. Hydrolysis of PLA–PGA polymers can be accelerated by an acidic environment as reported by Vert and colleagues,[44,45] who also determined that thick implants can develop a hollow core due to enhanced local degradation caused by trapped acidic by-products. In the present study the cone-shaped central hollow core found extending from the bottom of the implants under nonloaded conditions is indicative of this autocatalytic phenomenon. The hollow core does not extend to the top of the implant because the degradation products are able to diffuse out freely at that surface, preventing an accumulation of acid. However, the diffusion of by-products at the center and bottom of the implant is dependent on slowed diffusion through the constrained sides, so the acid by-products accumulate and cause accelerated breakdown of the molecular chains.

The hollow core is not evident in the dynamically loaded implants possibly because fluid flow is increased by loading, thus providing greater diffusion of the degradation products. Additionally, the formation of a hollow core or other signs of accelerated degradation in the center of the loaded specimens are likely to be obscured by the deformation imposed by dynamic loading. Vert et al.[44] reported the formation of hollow cores in PLA–PGA implants in an *in vivo* study. This observation may have a significant impact on the design of devices that are meant for load bearing applications or serve as scaffolds for tissue growth. Heterogeneous degradation can adversely affect the function and effective life of these implants as well as surrounding tissue, due to a loss in mechanical properties or retardation of tissue growth due to an acidic environment. Therefore, for PLA–PGA implants, there is a need to identify a critical size threshold below which the function and reliability of the implant will not be compromised due to heterogeneous degradation.

The results of this study support the methodology for evaluating *in vitro* degradation of biodegradable implants as a preliminary substitute for *in vivo* testing. Dynamic loading substantially increases protein release and also significantly increases the axial surface stiffness of the implant compared with nonloaded conditions. The implants used here will be subjected to mechanical compressive loads under *in vivo* conditions during rehabilitation. Thus, it is essential that *in vitro* degradation studies of these or similar implants include a dynamic functional environment.

ACKNOWLEDGMENTS

This study was sponsored in part by the Center for the Enhancement of the Biology and Biomaterials Interface (CEBBI) at The University of Texas Health Science Center at San Antonio, and an Alpha Omega Alpha Student Research Fellowship. We would also like to thank Jon Oeffinger, Dan Lanctot, George Constantinides, and Chong-Fang Zhu of the Orthopaedic Biomechanics Laboratory at The University of Texas Health Science Center at San Antonio for their help in conducting various parts of this study.

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